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Specificity of Nitroglycerin Assays: A Response

Keyphrases □ Nitroglycerin—analysis, stability, various procedures compared □ Vasodilators, coronary—nitroglycerin, stability analysis, various procedures compared □ Stability—nitroglycerin, analysis, various procedures compared

To the Editor:

This response will attempt to clarify several points raised by Morrison and Fung (1) concerning a report by Suphajettra *et al.* (2), which had discussed the possible formation of an interaction compound or complex between nitroglycerin in solution and polyethylene glycol 400. It was then suggested (2) that a loss of nitroglycerin "stability" had occurred due to the sequestering effect on it, which prevented its analysis by both UV (3) and colorimetric (4) techniques. As a result, the different apparent nitroglycerin degradation rates due to polyethylene glycol 400 (Fig. 1) as determined by these two methods simply reflected the relative recoveries of intact nitroglycerin or the "assayable" compound, attributable on one hand to "the more drastic hydrolysis procedure employed in the Bell method, which resulted in a relatively greater breakdown of the reaction compound . . ." (2). It was not our intention to imply that a basic deficiency existed with the UV–kinetic assay for determining free nitroglycerin molecules.

Furthermore, the term stability was used generically in the context of the report to describe not only degraded molecules but also those firmly bound or sequestered and, consequently, not available for analysis. Inasmuch as colorimetric nitroglycerin determination depends on the successful breakdown, in some stoichiometric fashion, of the molecule to a decomposition product, *i.e.*, nitrites, this method obviously cannot be regarded as stability indicating. It is precisely because of such a premise that the formation of an interaction compound, which effectively sequestered nitroglycerin from analysis by these two methods, was proposed. Furthermore, had no nitroglycerin "complex" been formed, the presence of nitrite ions, regardless of source (*i.e.*, as a chemical contaminant or as a nitroglycerin decomposition product), would produce linear curves closely parallel to the abscissa (Fig. 1) following the Bell colorimetric assay.

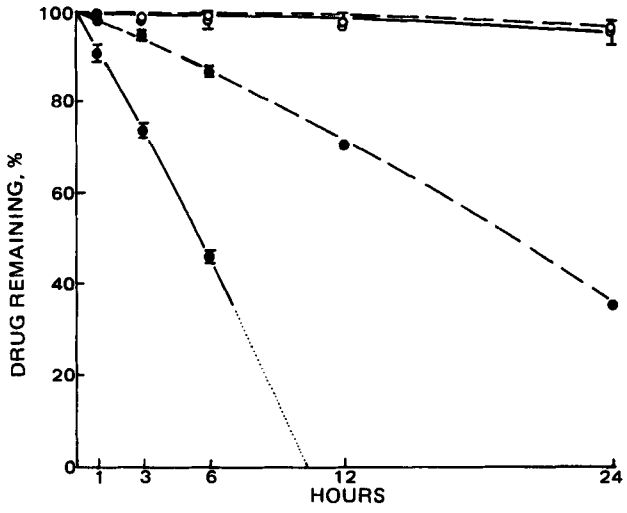

Figure 1—*Percentage of nitroglycerin remaining in povidone* (○) *and polyethylene glycol 400* (●) *solutions as a function of time at 80 ± 0.5° compared using the spectrophotometric* (—) *and colorimetric* (– –) *methods.*

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Percutaneous Butyrolactone Absorption in Rats

Keyphrases □ Butyrolactone—percutaneous absorption, with and without depilation, rats □ Hypnotics—butyrolactone, percutaneous absorption, with and without depilation, rats

To the Editor:

Butyrolactone (I) is a relatively nontoxic hypnotic agent when administered intravenously and orally to rats (1, 2). When I is introduced into the systemic circulation, it is instantaneously and completely converted to γ-hydroxybutyric acid (II). The latter is an endogenous substance in

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the brain (3) and has been used therapeutically as an intravenous anesthetic (4), particularly in pediatric and obstetric procedures.

Recently, DiVincenzo *et al.* (5) showed that a neurotoxic ketone, methyl butyl ketone, was absorbed through intact skin. Because II is subjected to extensive first-pass metabolism and has a short terminal biological half-life ($t_{1/2}$ $\simeq$0.5 hr in humans and rats), it is of interest to determine whether percutaneous I absorption is extensive and prolonged enough to provide sustained hypnosis. Furthermore, because I has physicochemical properties similar to other dipolar aprotic solvents such as dimethyl sulfoxide and thus might promote drug absorption through the skin, the absorption characteristics of I itself need to be determined.

The purpose of this study was to examine percutaneous I absorption. The rat was used as the experimental model because the pharmacokinetics of I were examined previously in this animal (1, 2) and because similar percutaneous absorption studies were carried out with another liquid (6, 7). Percutaneous I absorption was studied with and without a depilating agent since many percutaneous absorption studies in animals employ such an agent and the effect of depilating agents on skin permeability is not well documented.

Male Sprague–Dawley rats, 275–525 g, were prepared as previously described (6, 7). Absorption studies were conducted on the shaved abdomens of the animals over a 3 × 3-cm area. Compound I[1] was applied undiluted at a dose of 6.34 mmoles/kg (equivalent to 800 mg of II/kg) either over untreated skin (Topical Treatment A) or after treatment with 4 ml of thioglycolic acid-based depilating agent[2] (Topical Treatment B). The hair remover was left in contact with the shaved skin for 12 min and was then removed with wet gauze sponges; the skin was allowed to dry in air for 10 min before I application.

The animals were kept under light ether anesthesia, when required, throughout the experiments, and blood (~0.5 ml) was collected at selected intervals *via* the tail vein. Plasma II concentrations were determined according to a previously described procedure (8) and compared to those obtained from separate animals after intravenous and oral administration of the same dose (1, 2). When I is administered systemically, conversion to II is complete and no intact I can be measured.

Figure 1 shows the mean ($\pm SE$) plasma II concentrations obtained after the various routes of administration. Both intravenous and oral I doses gave relatively high plasma II concentrations, which were maintained above 2000 μg/ml for 2 hr after dosing. In contrast, when equimolar doses of I were applied topically, the mean plasma concentrations were <200 μg/ml during the whole 4-hr period. Comparison of the plasma concentrations after the two topical treatments showed that I absorption was more rapid and less variable after hair removal with a depilating agent. Statistical comparison using the two-tailed t-test showed that the concentrations obtained at the first two time points after dosing were significantly higher ($p < 0.02$) after pretreatment with the hair remover. Peak concentrations were observed within 0.5–1 hr after dosing

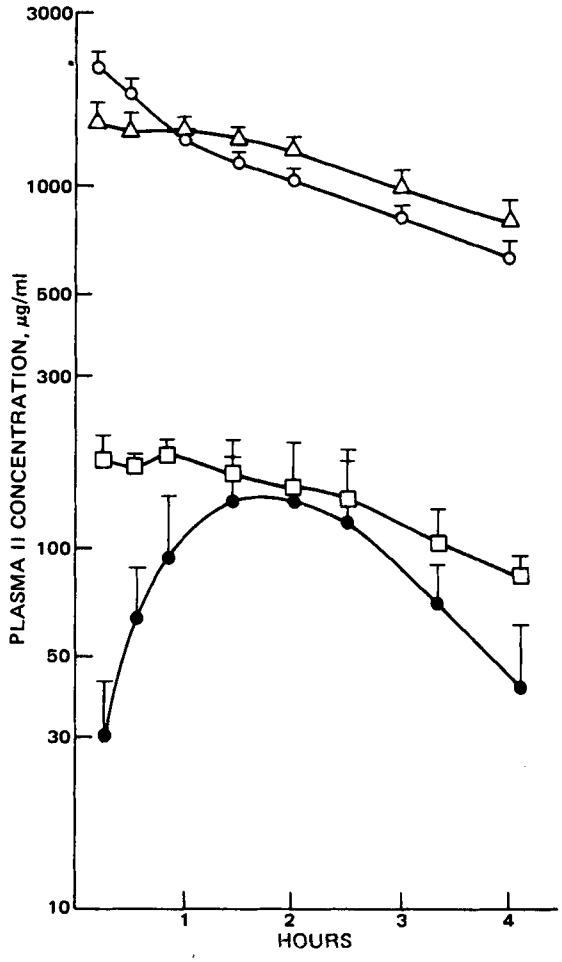

Figure 1—*Plasma II concentrations (mean ± SE) after 6.34 mmoles of I/kg administered through different routes. Key: ○, intravenous; △, oral; □, percutaneous, with hair remover; and ●, percutaneous, without hair remover.*

when a depilating agent was used and at about 1.5–2 hr in controls.

Table I gives the values of the area under the plasma concentration–time curve from 0–4 hr (AUC_{0-4}) for each treatment. Statistical comparison using the Tukey t-test (9) showed that $AUC_{0-4,iv}$ and $AUC_{0-4,po}$ were statistically different ($p < 0.01$) from those obtained after both percutaneous treatments, although $AUC_{0-4,iv}$ did not differ statistically from $AUC_{0-4,po}$ and the two values from the percutaneous treatments also did not differ from each other ($p > 0.05$).

A relative AUC_{0-4} value, defined as $AUC_{0-4,treatment}/AUC_{0-4,iv}$, also was determined for each treatment. In this case, the relative AUC_{0-4} values represent minimum estimates of the fraction of the dose absorbed, F, for the

Table I—Mean ± *SE* Values of the Area under the Plasma Concentration–Time Curve (0–4 hr) after Various I Treatments at 6.34 mmoles/kg

Treatment	$AUC_{0-4} \times 10^{-3}$, μg hr/ml	Relative AUC_{0-4}[a]
Intravenous	4.81 ± 0.37 (3)[b]	1.00
Oral	4.71 ± 0.38 (4)	0.98
Topical (A)	0.34 ± 0.18 (4)	0.07
Topical (B)	0.54 ± 0.08 (3)	0.11

[a] Relative to mean value obtained after intravenous administration. [b] Number in parentheses is the number of animals studied in each group.

[1] Eastman Organic Chemicals, Rochester, N.Y.
[2] Neet lotion hair remover, Whitehall Laboratories, New York, N.Y.

following reasons.

1. Capacity-limited elimination of II occurs at plasma concentrations above 400 μg/ml (2). Both intravenous and oral administrations at 6.34 mmoles/kg gave plasma concentrations well above this threshold, while percutaneous applications of I gave plasma concentrations below this concentration. When capacity-limited elimination occurs, the AUC increases disproportionally with respect to dose. The presence of capacity-limited elimination after the intravenous dose in the present case artifactually led to a larger $AUC_{0\text{-}4,iv}$ value for comparison with values obtained for the percutaneous treatments. Thus, the relative $AUC_{0\text{-}4}$ values for the percutaneous absorption experiments are likely to underestimate F.

2. Percutaneous drug absorption is sometimes fairly protracted. Some I absorption might still take place after the 4-hr period monitored in the percutaneous experiments. This factor would also lead to underestimation of F using the relative $AUC_{0\text{-}4}$ values. The present data could not be used for calculation of AUC from time zero to infinity because significant uncertainties in elimination $t_{1/2}$ estimates are produced by nonlinear elimination kinetics and possible incomplete absorption after the percutaneous doses.

The present results, however, suggested that at least ~10% of the percutaneous I dose was absorbed. Mean plasma II concentrations up to 200 μg/ml were obtained following a 6.34-mmoles/kg topical dose. These concentrations approach those levels (~400 μg/ml) required for complete hypnosis in the rat. In fact, some animals in the percutaneous experiments were sufficiently sedated that continued ether anesthesia was unnecessary. It is clear that the pharmacological effects of I can be elicited through skin absorption.

Hair removal by a commercial depilating agent apparently increased the rate of percutaneous I absorption in the rat. Although hair removal by shaving was done as close to the skin as possible, the remaining hair stubs probably still posed a significant barrier for drug absorption. On the other hand, the depilating agent might have caused some damage to the skin epidermis. This methodological uncertainty is encountered whenever haired animals are used for skin absorption experiments.

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Theophylline Analysis by Reversed-Phase High-Pressure Liquid Chromatography: Elimination of Interferences

Keyphrases ◻ Theophylline—analysis, reversed-phase high-pressure liquid chromatography, elimination of ampicillin and 1,7-dimethylxanthine interference ◻ Smooth muscle relaxants—theophylline, reversed-phase high-pressure liquid chromatographic analysis, elimination of ampicillin and 1,7-dimethylxanthine interference ◻ High-pressure liquid chromatography—analysis, theophylline, elimination of ampicillin and 1,7-dimethylxanthine interference

To the Editor:

High-pressure liquid chromatography (HPLC) has been applied to theophylline analysis in biological fluids (1–5). HPLC methods have distinct advantages over traditional UV spectrophotometric methods (6, 7) such as reduced sample requirement, greater specificity, and shorter analysis time. However, several drugs and drug metabolites interfere in the HPLC analysis of theophylline (8–11). Of particular concern are the interferences caused by ampicillin, a commonly used antibiotic, and by 1,7-dimethylxanthine, an important metabolite of caffeine. 1,7-Dimethylxanthine is difficult to detect using ratios of peak heights obtained at two different wavelengths because of the similarity of its UV spectrum to that of theophylline. We have found elevation of serum theophylline values caused by 1,7-dimethylxanthine to be as high as 2 μg/ml. Such a discrepancy could cause misleading interpretation of pharmacokinetic data and would lead to inaccurate dosage regimen calculations.

We modified the method of Orcutt *et al.* (2) to eliminate interferences caused by ampicillin and 1,7-dimethylxanthine by using either a reversed-phase column with a 5-μm support or a radial compression module with a reversed-phase cartridge. A mobile phase of methanol–tetrahydrofuran–sodium acetate buffer provided the desired resolution.

The HPLC system was composed of a single pump[1], a loop injector[2], and a dual-wavelength UV detector with absorbance capability at 254 and 280 nm[3]. A precolumn[4] helped prolong column life. Two types of prepacked columns were used. One consisted of a steel, 25 × 0.5-cm column packed with octadecylsilane bonded to a 5-μm irregular support[5]. The other, intended for use in a radial compression unit, was a polyethylene column packed with octadecylsilane bonded to a spherical 10-μm silica support[6].

[1] Model 6000A, Waters Associates, Milford, Mass.
[2] Model U6K, Waters Associates, Milford, Mass.
[3] Model 440, Waters Associates, Milford, Mass.
[4] Co:Pell ODS, Whatman, Clifton, N.J.
[5] Partisil PXS 5/25 ODS, Whatman, Clifton, N.J.
[6] RCM with radial pak A, Waters Associates, Milford, Mass.

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